

Emerging Resistance in Gram-Negative Bacteria – CLSI Recommendations

James H. Jorgensen, PhD

Most Important Emerging or Evolving Resistance in GNs

- ✓ Newer ESBLs
- ✓ Plasmid-mediated AmpCs
- ✓ KPCs,
- ✓ VIM, IMP
- ✓ **NDM**
- ✓ *Acinetobacter*, *Stenotrophomonas*

Most Recent CLSI AST Documents

- ✓ M2-A10 (2009) - disk diffusion
- ✓ M7-A8 (2009) - aerobic MIC
- ✓ M100-S20 (2010) - tables for M2 & M7
- M100-S10U
- ✓ M45-A2 (2010) infreq or fastid bacteria
- ✓ M11-A8 (2009) - anaerobe AST
- ✓ M39-A3 (2009) - preparation of antibiograms

New Table Formats in M100-S19 and –S20

- ✓ Tables 2A-2L include both MIC and zone diameter interpretive criteria in parallel columns appropriate to the organisms in the table
- ✓ Where disk testing is not appropriate, the disk columns are left blank
- ✓ Relevant comments for MIC or disk tests have been extracted and condensed or modified where needed for the new tables
- ✓ Appendixes have moved from the back to go with the relevant Tables

Extended Spectrum Beta-Lactamases

- ✓ “Classical ESBL” - mutations of TEM or SHV plasmid-mediated enzymes normally found in *E. coli* and *Klebsiella*
 - Now TEM-1 to 182, SHV-1 to 134 (as of 8-20-10) - Source: www.lahey.org/studies/webt.asp
- ✓ Hydrolyze 3rd and 4th gen cephs and aztreonam at high bacterial inoculum in vitro, but not cephamycins or carbapenems
- ✓ Differences in substrate specificity -especially ceftaz vs. cefotax
- ✓ Mostly cause healthcare-associated infections

Normal Hosts for TEM-1 and SHV-1

v TEM-1

- $\geq 60\%$ of *E. coli* (amp resistance)
- $\sim 40\%$ of *Haemophilus* (amp resistance)
- *N. gonorrhoeae* (PPNG)
- Some other species of *Enterobacteriaceae*

v SHV-1

- $> 99\%$ of *Klebsiella pneumoniae* (amp resistance)

*To review briefly Classical ESBL
background.....*

Molecular Basis of ESBLs

<i>Enzyme</i>	<i>Ceftaz</i>	<i>Amino Acid Position</i>		
		<i>MIC</i>	<i>104</i>	<i>164</i>
TEM-1	0.12	Glu	Arg	Glu
TEM-10	> 256	Glu	<i>Ser</i>	<i>Lys</i>
TEM-12	16	Glu	<i>Ser</i>	Glu
TEM-26	256	<i>Lys</i>	<i>Ser</i>	Glu

from: Jacoby, IDCNA 11:875, 1997

Different Substrate Affinities of ESBL

Enzyme	MICs		
	<i>Ceftaz</i>	<i>Cefotax</i>	<i>Aztreo</i>
TEM-1	0.12	0.06	0.12
TEM-10	> 256	1	128
TEM-12	16	0.12	1
TEM-26	256	0.5	64

from: Jacoby, IDCNA 11:875, 1997

Inoculum Effect with ESBLs - MICs with SHV-3 producing *C. freundii*

	Inoculum	
	10^5	10^7
Cefotaxime	2	256
Ceftazidime	1	32
Cefepime	0.5	>128
Meropenem	0.06	0.06

Thomson, AAC45:3548, 2001

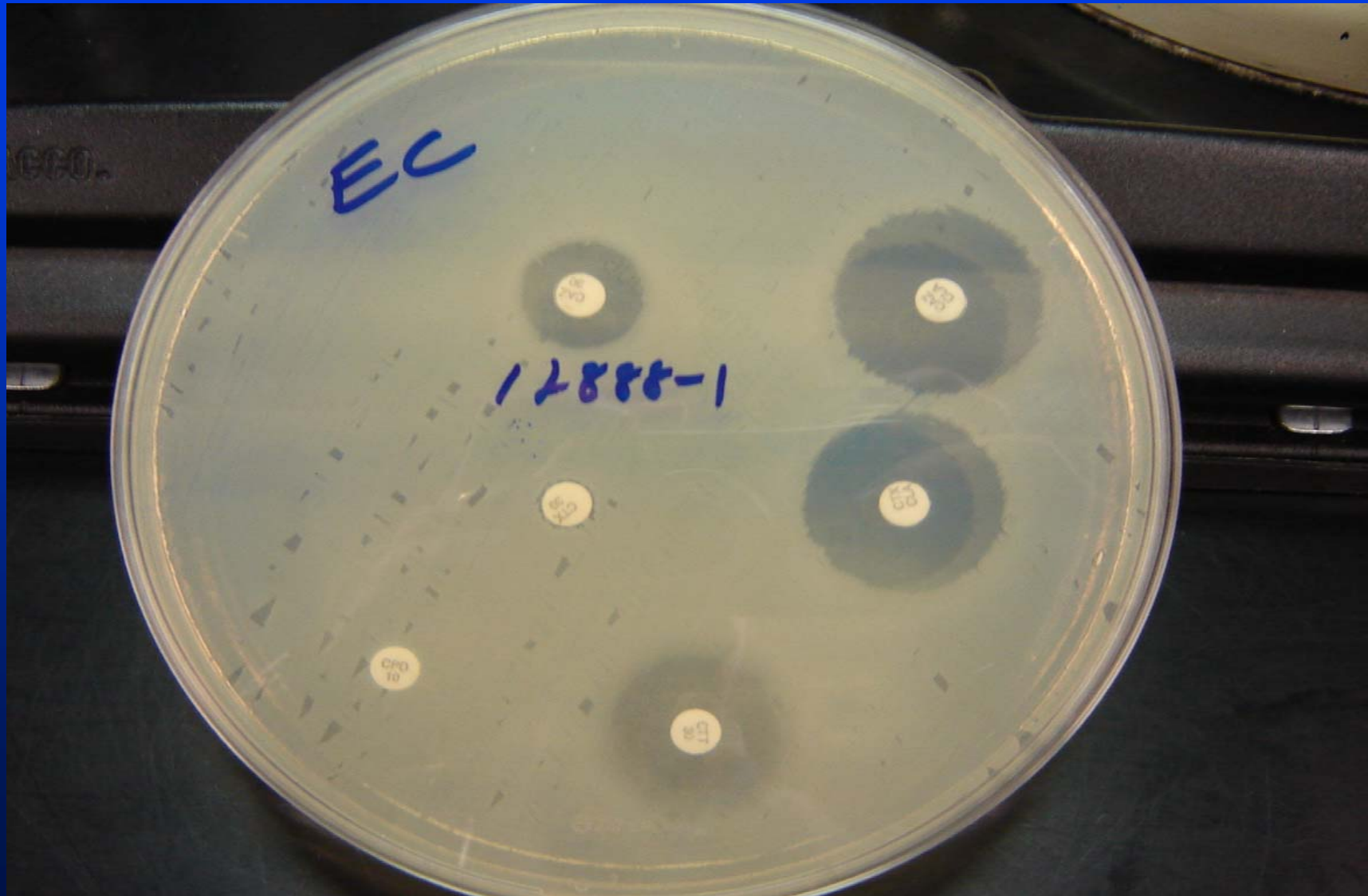
Gram-Negative Species Known to Harbor ESBL

- ✓ *Klebsiella pneumoniae*
- ✓ *Klebsiella oxytoca*
- ✓ *E. coli*
- ✓ *Proteus mirabilis*
- ✓ *Salmonella* spp.
- ✓ Also in *Citrobacter*, *Enterobacter*,
Serratia, *Morganella*, *P. aeruginosa*

ESBL That Are Not Derived From TEM or SHV; The CTX-M enzymes

- ✓ CTX-M-1 thru 100 - often hydrolyze cefotaxime better than ceftazidime
- ✓ Derived from *Kluyvera ascorbata*
 - Chromosomal gene now on conjugative plasmids
- ✓ **Most common ESBL** in Latin America, Asia, Europe, UK, and now the U.S. (SA, Pittsburgh, Philadelphia, and 2 national surveillance studies)

E. coli with “Cefotaximase”

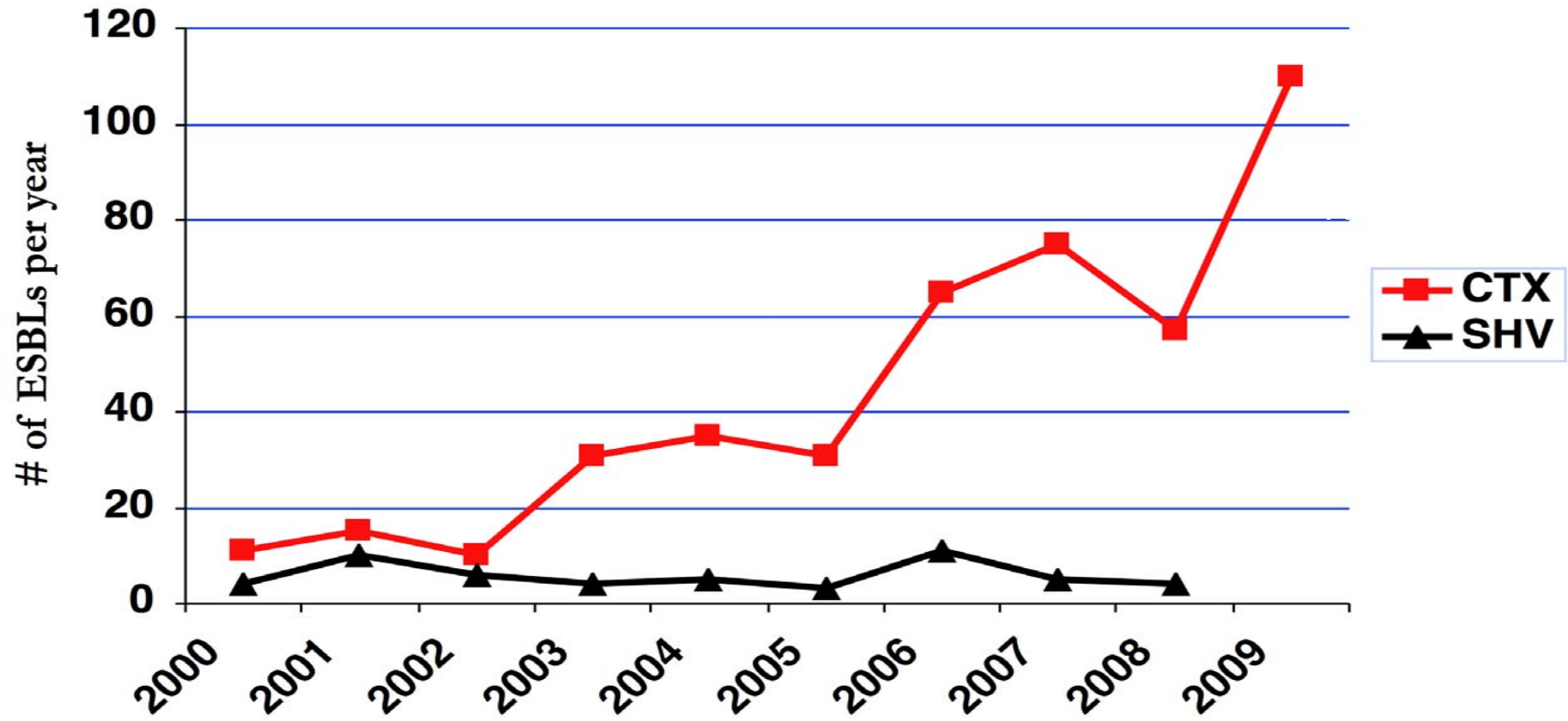


Assessment of ESBLs in San Antonio

- v Retained ESBL isolates from 2000 - 2008
- v PCR and sequencing for CTX-M, SHV, TEM
- v CTX-Ms emerged as predominant ESBL
 - in *E. coli*, *K. pneumoniae*, *K. oxytoca*, *P. mirabilis*, *Enterobacter* spp., *M. morganii*
 - Predominantly CTX-M15 in *E. coli*, often from outpatient urines
- v 86% fluoroquinolone resistant (100% *E. coli*); 66% to SXT

– Lewis, et al, AAC 51:4015, 2007

Increasing Numbers of ESBLs



CTX-M UTIs in San Antonio

- v 40 patients with CTX-M *E. coli* UTI between 2006 and 2008
 - Mean age 61 (22-84)
 - 70% female
 - 75% outpatient infections
 - 15% lived in or had HC in Mexico
 - 18% no HC contact in prev. 6 mos., no recognized comorbidities and younger (43)
 - Lewis, et al 2009 ICAAC

CTX-M UTIs in San Antonio (cont)

- v 22/30 outpatients treated with a fluoroquinolone
 - 97% of isolates resistant
 - 59% failed clinically or microbiologically
 - OPs that received fosfomycin, nitrofurantoin, or SXT (when suscept) responded favorably
- v 6/9 inpatients that received a FQ, cefepime, or pip-tazo failed
 - Those treated with a carbapenem, trim-sulfa, or gent (when suscept) responded favorably
 - Lewis, et al 2009 ICAAC

CTX-M ESBL in the ST131 clone of *E. coli* is geographically dispersed

- v ST131 (O25:H4) producing CTX-M in the U.S.
 - Chicago, Portland ME, **San Antonio**, and Seattle
 - Johnson, et al, Abs ICAAC/IDSA 2008
- v ST131 producing CTX-M14 and 15 in Spain
 - Possible source in LTCFs
 - Blanco, et al, JAC 63:1135, 2009
- v **ST131 is a fluoroquinolone-resistant pandemic clone** that has acquired a conjugative plasmid with CTX-M ESBLs

Is the Source of CTX-Ms our Food Supply?

- v CTX-M1 and CTX-M15 in clinical isolates of *E. coli* from food producing animals in France

- Cattle, swine, poultry
- Recognized because of use/resistance to ceftiofur
- CTX-M9 also in *Salmonella* from poultry

Meunier, et al, Int J Antimicrob Agents 28:402, 2006

- v CTX-Ms in broiler chickens in Belgium

Smet, et al, AAC 52:1238, 2008

- v CTX-M2 in *E. coli* from cattle in Japan -assoc. with ceftiofur use

Shibata, et al, Emerg ID 10:69, 2004

- v CTX-M15 producing *E. coli* from raw chicken sold at a Pittsburgh area supermarket

Doi, et al, Emerg ID 13:1121, 2007

CTX-M ESBL in the ST131 clone of *E. coli*; In the food supply?

- v ST131 (O25:H4) producing CTX-M in the U.S. and Spain
 - Johnson, et al, Abs ICAAC/IDSA 2008
 - Blanco, et al, JAC 63:1135, 2009
- v ST131 is a fluoroquinolone-resistant pandemic clone that has acquired a conjugative plasmid with CTX-M (sometimes with *qnr* and AAC6'-Ib-cr)
- v CTX-M producing *E. coli* has been recovered from Chicken, pork, and beef in France, Belgium, Japan, and the U.S.
 - Meunier, et al, Int J Antimicrob Agents 28:402, 2006; Smet, et al, AAC52:1238, 2008; Shibata, et al, Emerg ID 10:69, 2004 Doi, et al, Emerg ID 13:1121, 2007

CTX-M ESBLs and UTIs

- ✓ Increasing incidence of outpatient urinary isolates with CTX-M
 - Usually multi-drug resistant (FQs, SXT)
 - Empiric outpatient treatment may require rethinking
 - Urine cultures needed more often
 - Ultimately need better rapid diagnostic testing and new antimicrobials

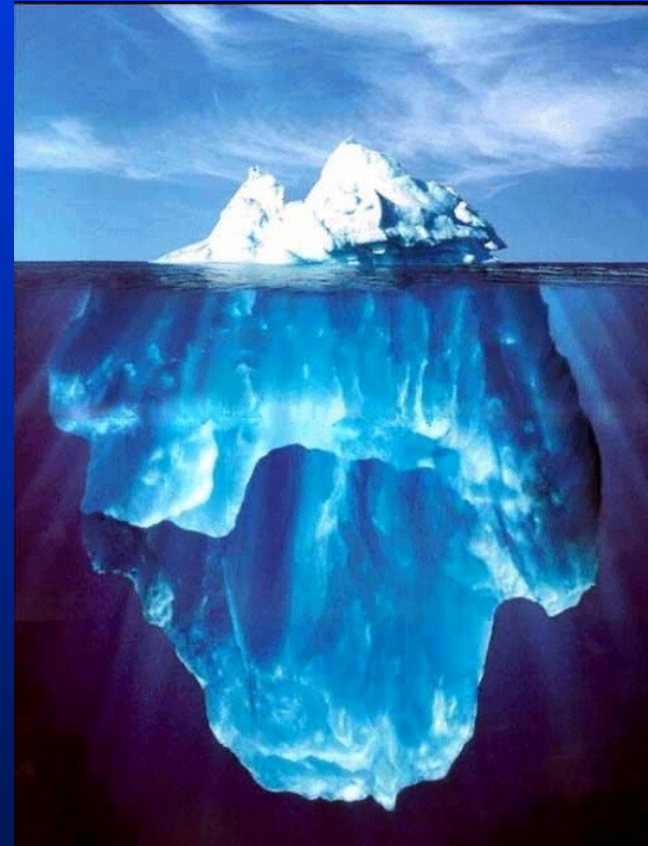
What are the Options for OP Therapy of UTI with CTX-M *E. coli*?

	% Susceptible
Fosfomycin	91.3
Cefdinir + Amox-clav	89.1
Nitrofurantoin	73.9
Doxycycline	10.9
Ciprofloxacin	4.3
Ertapenem	100

Testing of 46 isolates - Prakash, et al, AAC 59:1278, 2009

Looking beneath the surface of...

- v If Labs do not screen urinary isolates for ESBLs.....
- v If physicians do not order urine cultures, at least from recurrent infections.....



Two Step Process of Detection and Confirmation of ESBLs

- v Test “indicator” drugs with special “screening” breakpoints
 - cefpodoxime or look for elevated MICs of **ceph 3s**
- v Must confirm with clavulanate combos of cefotaxime **and** ceftazidime by MIC or disk
- v Report as ESBL if either clavulanate combo is positive
- v Works with both “classical” and CTX-M ESBLs

**Klebsiella pneumoniae
ATCC 700603**



**CAZ
30**



**CAZ/Clav
30/10**



**CTX
30**



**CTX/Clav
30/10**

Previous Laboratory Reporting of ESBL-Producing Isolates

- ✓ “Expertize” results to resistant for all penicillins, aztreonam, and “true cephalosporins” irrespective of individual test results and/or
- ✓ Provide a warning comment that ESBL-producers should be considered clinically resistant to all penicillins, cephalosporins, and aztreonam

Other Causes of Resistance* to ES Cephalosporins in *Klebsiella* and *E. coli*

- v Plasmid-mediated AmpC
- v Production of multiple beta-lactamases
- v Porin (OMP) mutations

* Clavulanate-negative

AmpC Beta-Lactamase

- v *ampC* gene is present in all *Enterobacter*, *Citrobacter freundii*, *Morganella morganii*, *P. aeruginosa*
 - Resistance to all cephs except cefepime
- v Selection of resistant mutants with “up-regulated” production of ampC during therapy
- v *ampC* can be plasmid-mediated in some *E. coli* and *K. pneumoniae*
 - Jacoby and Munoz-Price, NEJM 352:380, 2005

Plasmid-Mediated AmpC

- v Plasmid mediated version of *Enterobacter* chromosomal enzyme
- v In *E. coli*, *Klebsiella*, *P. mirabilis*, *Salmonella*
- v CMY, FOX, ACC, DHA
 - CMY-2 in some CA *E. coli* UTIs
 - Most are nosocomial

Jacoby, CMR 22:161, 2009

ESBL vs. Plasmid-AmpC

ESBL

- v Susceptible to cephamycins
- v Inhibited by clavulanate
- v Can hydrolyze cefepime at high inoculum

AmpC

- v Resistant to cephamycins
- v Not inhibited by clav
- v Hydrolyzes cefepime poorly

Boronic Acid Inhibition of AmpC



The *New* CLSI Approach

- ✓ To avoid confusion among microbiologists and clinicians, AND because of the growing complexity of resistance mechanisms
 - Change (lower) cephalosporin breakpoints for members of the *Enterobacteriaceae* !!!!
- ✓ Used pharmacodynamic principles to derive new breakpoints

Cephalosporin* Breakpoints Differ by Standards Organizations

	Susceptible Breakpoint ($\mu\text{g/ml}$)
U.S. (CLSI)	≤ 8
EUCAST	≤ 1
France (CA-SFM)	≤ 4
Sweden	≤ 4
Norway	≤ 2
England (BSAC)	≤ 1
Spain (MENSURA)	≤ 1

* cefotaxime, ceftriaxone, ceftazidime

New Breakpoints Published in January, 2010

	S	I	R	
Cefazolin		1	2	4
Cefotaxime	1	2	4	
Ceftriaxone	1	2	4	
Ceftizoxime	1	2	4	
Ceftazidime	4	8	16	
Cefepime	8	16	32	
Aztreonam	4	8	16	

Advantages/Disadvantages of Proposed Breakpoints

v Advantages

- Eliminate or reduce need for routine ESBL screening and reporting?
- Increased patient safety ?
- Rare failures with ESBL with MICs $\leq 2 \mu\text{g}$

v Disadvantages

- Conflict with FDA-approved drug PIs
- Conflict with FDA-cleared device breakpoints
- Period of paralysis while devices undergo revision and new FDA clearance (2-3 yrs?!)

What to do during the “interim” period

- v It may take diagnostic companies 2-3 years to obtain clearance for new cephalosporin breakpoints for enterics
- v In the meantime, labs may continue to test and report ESBL producers in the “old” manner
- v Disk users can adopt new breakpoints now

Be prepared for:

- v ESBL producers that were previously “expertized” to cefepime and ceftazidime resistance to now be reported as “susceptible”
- v Antibigrams will change
 - Cefepime suddenly looks better

The *Newest* Mechanisms of Concern - *Carbapenemases*

- v An alphabet soup of rapidly emerging carbapenemases
 - KPCs 1-10
 - IMP 1-24
 - VIM 1-22
 - Some OXA enzymes - esp. in *Acinetobacter*
 - *and now the NDM*

Source: www.lahey.org/studies/webt.asp

KPC Carbapenemases

- v Plasmid-mediated - KPCs
 - *Klebsiella pneumoniae* carbapenemases can hydrolyze all beta-lactams
 - Since first discovered in NC (Yigit, AAC 2001) have rapidly spread in NY, NJ, PA
 - Most often in ICU patients, devices, caths
- v Have also been found in Puerto Rico, Israel, France, Greece, Scotland, Colombia, China
 - Bradford, et al, Clin Infect Dis 39:55, 2004
 - Nordmann, Lancet ID, 9:228, 2009

KPCs (cont)

- ✓ Most have been in *K. pneumoniae* or *K. oxytoca*
 - *Enterobacter* second most common
 - Now emerging in *E. coli* with CTX-M
 - Also in *P. mirabilis*, *Citrobacter*, *Serratia*, *Salmonella*, *P. aeruginosa*, and *P. putida*
- ✓ Most often resistant to other non-Beta-lactams - XDR phenotype
- ✓ May be difficult to detect by standard lab methods
- ✓ Demonstrates modest clavulanate effect (~3-4mm) and inhibition by boronic acid

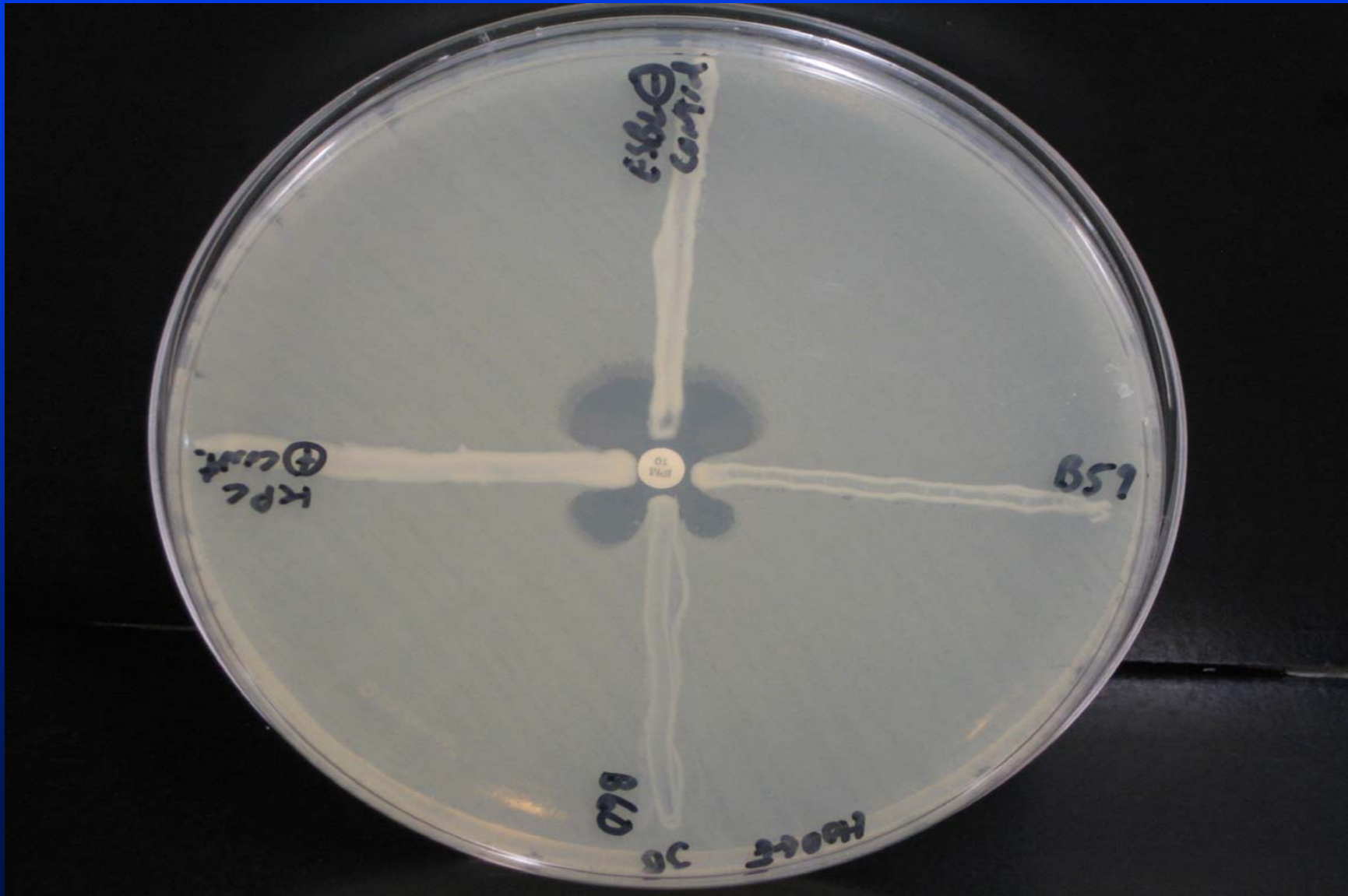
How Do Labs Perform in Detection of KPCs?

- v CAP sample DA-05, 2007 illustrated problems of detection
 - Partial clavulanate effect - like ESBL
 - Some commercial systems (and disks) had a high false susceptible rate with imipenem
 - Meropenem also problematic
 - Best detection by testing ertapenem
 - Ertapenem > meropenem > imipenem

CLSI Recommendations for Detection of KPCs in 2009-2010

- ✓ Note **elevated** carbapenem MICs of 2 or 4 µg/ml or diminished disk zones (19-21 mm ertapenem, 16-21 mm meropenem)
- ✓ Best to test both **ertapenem** and meropenem (Note: not usually high level R due strictly to KPCs)
- ✓ Confirm with modified Hodge test
- ✓ Add comment that infection with KPC may not respond to carbapenem therapy
- ✓ Report promptly to infection control

Modified Hodge Test



Modified Hodge Test

- ✓ Inoculate whole MHA plate with 1:10 dilution of 0.5 McF suspension of *E. coli* ATCC 25922
- ✓ Place ertapenem and meropenem disks on plate
- ✓ Streak loopful of growth of test and QA isolates from disk edge outward
- ✓ QA orgs - *K. pneumo* ATCC BAA 1705 (KPC-2) and *K. pneumo* ATCC BAA 1706 (carbapenemase neg)

Not all carbapenem resistance is due to KPCs

- ✓ 14 enteric isolates with carbapenem MICs ≥ 4
 - 9 *K. pneumo*, 3 *E. cloacae*, 2 *E. coli*
 - Isolates from two large teaching hospitals
- ✓ Modified Hodge tests and KPC and ESBL PCR on all isolates
 - 1 *E. cloacae* produced KPC-2
 - All produced at least 1 ESBL (CTX-M15, SHV-12 or 2A) plus porin (OMP) changes
 - 1 *E. coli* produced ampC plus porin changes

Bennett, et al, DMID, 2009

The Other Carbapenemases - Metallo-Beta-Lactamases

- v VIM and IMP most common
- v Require zinc for activity
- v Have been found mostly in *Pseudomonas* and *Acinetobacter* (and a few enterics)
- v Europe, Asia, S. America, N. America
- v Usually susceptible to **aztreonam** (but may be hidden by other enzymes)

New Delhi Metallo- β - Lactamase

- v First reported in 2009 from patient who traveled to India
- v Additional 183 isolates reported in 2010 from India, Pakistan, U.K., and U.S.
 - *K. pneumoniae*, *E. coli*, *Enterobacter*, *Proteus*, *Citrobacter*
 - NDM-1 carried on plasmids of various sizes

Yong, AAC 53:5046, 2009; Kumarasamy, Lancet 3099, 2010; Patel, 2010

New Delhi Metallo- β - Lactamase (cont)

- ✓ Resistance to all β -lactams and also FQs, aminoglycosides, tetracyclines
- ✓ 56-67% of isolates susceptible to tigecycline
- ✓ 89-100% susceptible to colistin
- ✓ **Outpatient UTIs** as well as healthcare infections
- ✓ A consequence of “medical tourism?”

Kumarasamy, Lancet 3099, 2010

The Newest Breakpoint Changes for GNs in 2010 (M100-S20 U, June)

v Lowering of the carbapenem BPs

	S	I	R
imipenem	1	2	4
meropenem	1	2	4
doripenem	1	2	4
ertapenem	0.25	0.5	1

The new carbapenem breakpoints
may obviate the need for the
Hodge test

Another MDR Gram-Negative - *Acinetobacter*

- v Generally low virulence GN water org.
- v Can cause wound, catheter, bloodstream infections - e.g., returning OEF or OIF wounded soldiers
- v Multiple resistance mechanisms including cell wall impermeability, efflux, AmpC, some ESBLs (SHV-5), OXA23, 24/40, 48, 51, and 58 carbapenemases, AACs

Acinetobacter Susceptibility Testing

- v “Trailing” endpoints and skip wells complicate MIC interpretations of imipenem and colistin
 - Heteroresistance ~ 1 in 10^6 cells more resistant
 - Hawley, et al, AAC 51:376, 2007
 - Swenson, et al, JCM 42:5102, 2004
- v **No FDA cleared devices for doing colistin MICs**; disks and E strips don't work (CMR 21:449, 2008)
- v No breakpoints for tigecycline
- v Testing combinations (colistin + rif, tigecycline + rif) not practical

Background

- ✓ FDA CDER mandated by the Federal Food, Drug & Cosmetic Act to approve official drug “labels” when new drugs are approved for use
 - Antibacterial agent labels have included a susceptibility testing section since ~mid-1970s
- ✓ FDA CDRH “clears” susceptibility testing devices through 510(k) premarket notification
 - Clearance requires that performance have “substantial equivalence” to a reference method (EA, CA, repro)
 - Clearance includes any interpretations made by device software
 - Requirements are specified in a guidance document

Breakpoints Used for Device Clearance by FDA

- v Until 2003, performance based upon category agreement with FDA label and NCCLS BPs “if based on more current recommendations for detecting organism resistance when resistant mechanisms were not recognized (or did not exist) during the FDA drug evaluation”
- v Starting in early 2005, FDA stated that only drug PI BPs be used for 510(k) clearances
- v FDAAA act of 2007 required FDA to update drug labels and make BP changes available as soon as known (w/i 30 days)

Examples of Out of Date or Incomplete Drug Labels

- ✓ At least 12 of 30 most commonly prescribed antibacterials have badly out of date labels
- ✓ Oxacillin - test methicillin by disk
- ✓ Ampicillin - only disk testing, no MIC BPs
- ✓ Gentamicin, amikacin - disk testing BPs only
- ✓ 9 other common drugs had incomplete or only general MIC BPs
- ✓ FDA later estimated that 70 of 100 labels needed updating

Going forward, the FDA may rely
on the CLSI to advise updated
breakpoints for older drugs

Only time will tell if this really happens